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13. ABSTRACT ***(Maximum 200 Words)***

Estrogen and progesterone, signaling through their cognate receptors (ER and PR, respectively), promote the growth of mammary glands via growth factors which signal through the family of erbB receptors, such as C-neu/erbB2. We have made the paradoxical observation that in transgenic mice over-expressing C-neu, in which mammary tumors arise, mammary growth is, in fact, compromised during puberty without any gross impairment in growth during pregnancy. Our hypothesis is that (a) the individual and combined effects of ER, PR and/or C-neu depends on the mammary epithelial sub-type and the interactions among these receptors, (b) the net outcome of these interactions is to direct the developmental fate of the various epithelial sub-classes and (c) a perturbation in these interactions, resulting from either an altered expression or signaling through these receptors leads to aberrant morphogenesis and neoplasia. Accordingly, we propose: (1) To examine the expression patterns of ER, PR and C-neu in mammary glands of wild type and C-neu transgenic mice during various developmental states and their relationships to cells undergoing proliferation; and, 2) To examine the growth patterns of mammary glands of C-neu transgenic mice upon serial transplantation into de-epithelialized fat pads. Our proposed studies will identify changes conducive to tumorigenesis, occurring in response to C-neu over-expression during early mammary development; this, in turn, can help to devise prophylatic strategies aimed at prevention, and hence, its significance.

14. SUBJECT TERMS
breast cancer, C-neu,erbB2, estrogen receptor, epithelial cells

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Introduction

Signaling by the sex steroids, estrogen and progesterone, through their cognate receptors, is essential for mammary gland morphogenesis. As such, ductal growth during puberty requires estrogen receptor alpha (ERα) and not progesterone receptor (PR) while lobular-alveolar growth during pregnancy requires PR. The growth promoting effects of these steroids are believed to be mediated by growth factors that signal through the family of erbB receptors, such as C-neu/erbB2. We have found that in transgenic mice over-expressing C-Neu (1), ductal growth during puberty is compromised without any gross impairment in lobulo-alveolar growth during pregnancy (2). Normal mammary glands consists of various epithelial subtypes and the distribution of ERα, PR and C-Neu are heterogeneous in the epithelium and appropriate signaling through hormones and growth factors require cell-cell interactions. Accordingly, we believe that (a) the individual and combined effects of ERα, PR and/or C-Neu (in conjunction with other erbB receptors) depends on the mammary epithelial sub-type and the interactions among these receptors and (b) the net outcome of these interactions is to direct the developmental fate of the various epithelial sub-classes towards ductal or lobular morphogenesis. To test this we are examining the expression patterns of ERα, PR and C-Neu in mammary glands of wild type and C-Neu transgenic mice during various developmental states and identifying the relationships between these expression patterns to cells undergoing proliferation.

Body

The tasks outlined in the approved statement of work are as follows:

(1) To examine the expression patterns of ER, PR and C-neu in mammary glands of wild type and C-neu transgenic mice during various developmental states and identify their relationships to cells undergoing proliferation; (2) To examine the growth patterns of mammary glands of C-neu transgenic mice upon serial transplantation.

(Although this proposal was approved for funding as of 07/01/01, the final agreement between DOD and LBNL was not completed and the funds were not released for research until February 2002. Therefore, all the research accomplishments described below cover only the period from February 2002 to September 2002 and pertain to Task 1).

To examine the expression patterns of ER and PR, immunolocalization studies were performed on either frozen mammary sections, using an indirect immunofluorescence assay, or paraffin embedded sections, using immunoperoxidase assay, as previously described (3-5). For detection of PR, we used the following antibodies: an anti-rabbit polyclonal antibody prepared against mouse PR generated by our laboratory and an anti-rabbit polyclonal antibody prepared against human PR, purchased from DAKO. For detection of ER, an anti-mouse monoclonal antibody prepared against human ER 6F11), purchased from Novocastra, was used.

Studies on immunolocalization of ERα did not reveal any significant differences in the intensity of immunostaining between the mammary glands of wild type and C-Neu transgenic mice either in pubertal or adult mice (Fig. 1, Panels A-D). However, in pubertal mice but not in adult, there was a decrease in the number of ERα-positive mammary epithelial cells in the C-Neu transgenic mice (Fig. 1E).

Studies on immunolocalization of PR, using two different antibodies and two different techniques, revealed that there were alterations in mammary glands of adult C-Neu transgenic mice. As shown in figures 2, 3 and 4, there was a decrease in the intensity of immunostaining of PR in mammary glands of C-Neu transgenic mice. In addition, there was also a decrease in the number of PR-positive cells in mammary glands of adult C-Neu transgenic mice (Fig. 5). Similar to adult mice, the decrease in PR, both with regard to level and number, was also evident in mammary glands of pubertal (6 weeks old) C-Neu transgenic mice (Fig.6)

Key Research Accomplishments

Overexpression of C-Neu leads to alterations in the expression patterns of PR in mammary glands of both pubertal and adult mice.

Reportable Outcomes:

None

Conclusions

Our studies, so far, have revealed that there are differences between the mammary glands of wild type and C-Neu mice with regard to their expression patterns of PR. The synthesis of PR in mammary epithelial cells is regulated by estrogen (6). As such, the levels of PR reflect both the degree of estrogen responsiveness in mammary epithelial cells and their potential to respond to progesterone. We find the alterations in PR expression in mammary glands of C-Neu mice as early as six weeks of age. Therefore, we propose that over expression of C-Neu leads to alterations in ovarian steroid hormonal regulation of mammary epithelial cells and represents a very early event in C-Neu dependent carcinogenesis.

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Appendices

Figure 1. Analyses of ERα expression in the mammary glands of wild type and C-Neu transgenic mice.

Figure 2. Analyses for PR expression in mammary glands of adult wild type and C-Neu transgenic mice.

Figure 3. Analyses of PR expression in the mammary glands of adult wild type and C-Neu transgenic mice.

Figure 4. Analyses for PR expression in mammary glands of adult wild type and C-Neu transgenic mice.

Figure 5 Quantitative analyses of PR expression in mammary glands of adult wild type and C-Neu transgenic mice.

Figure 6. Analyses of PR expression in the mammary glands of pubertal wild type and C-Neu transgenic mice.

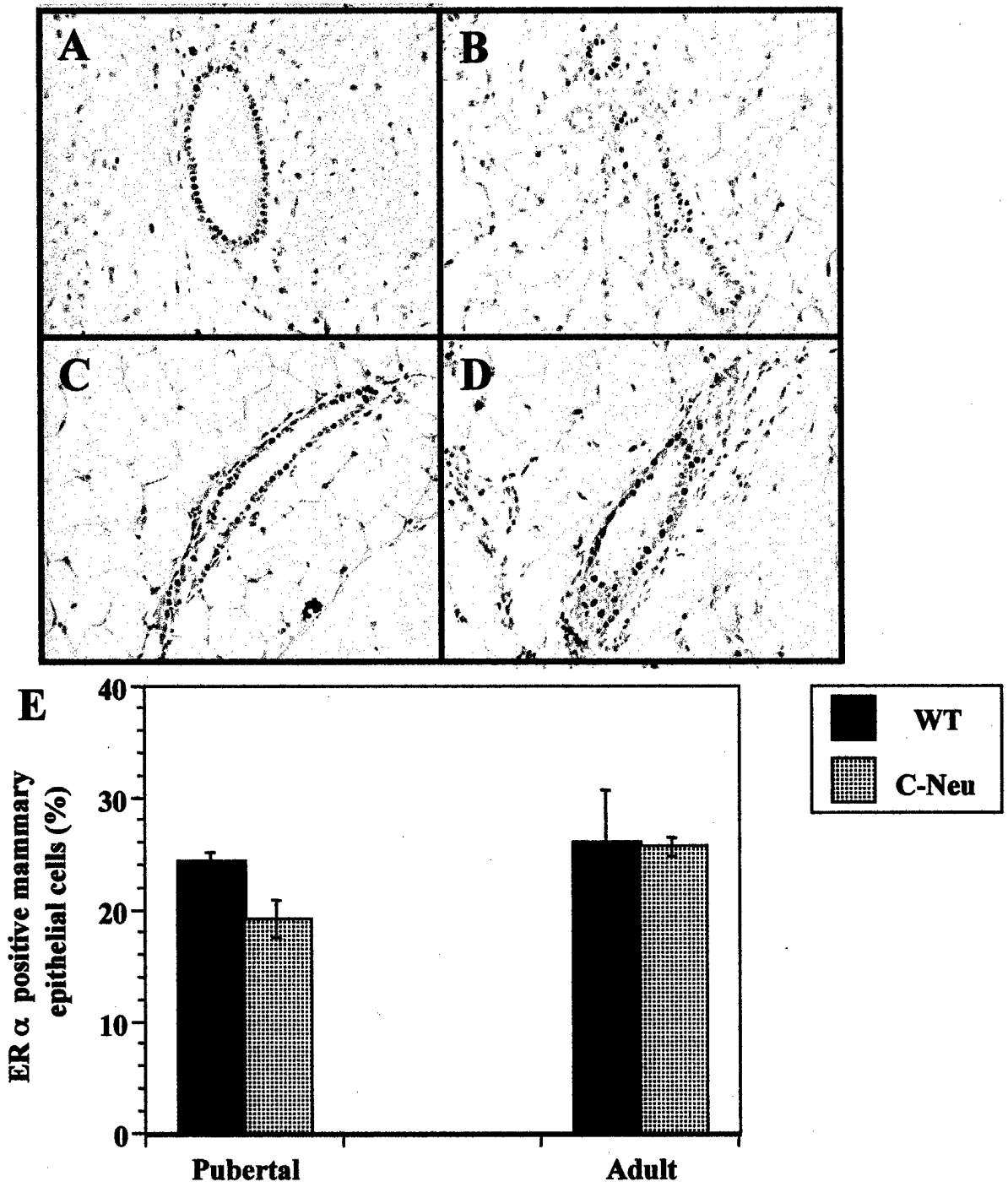

Figure 1. Analyses of ER α expression in the mammary glands of wild type and C-Neu transgenic mice. Panels A and C: pubertal (6-week-old) and adult wild type mice. Panels B and D: pubertal and adult C-Neu transgenic mice. Panel E: quantitative analysis of ER α-positive mammary epithelium in both wild type and C-Neu transgenic mice. The intensity of immunostaining appeared to be equivalent between the mammary glands of wild type and C-Neu transgenic mice. However, there was a reduction in number of ER α-positive cells in pubertal but not adult C-Neu transgenic mice (Panel E).

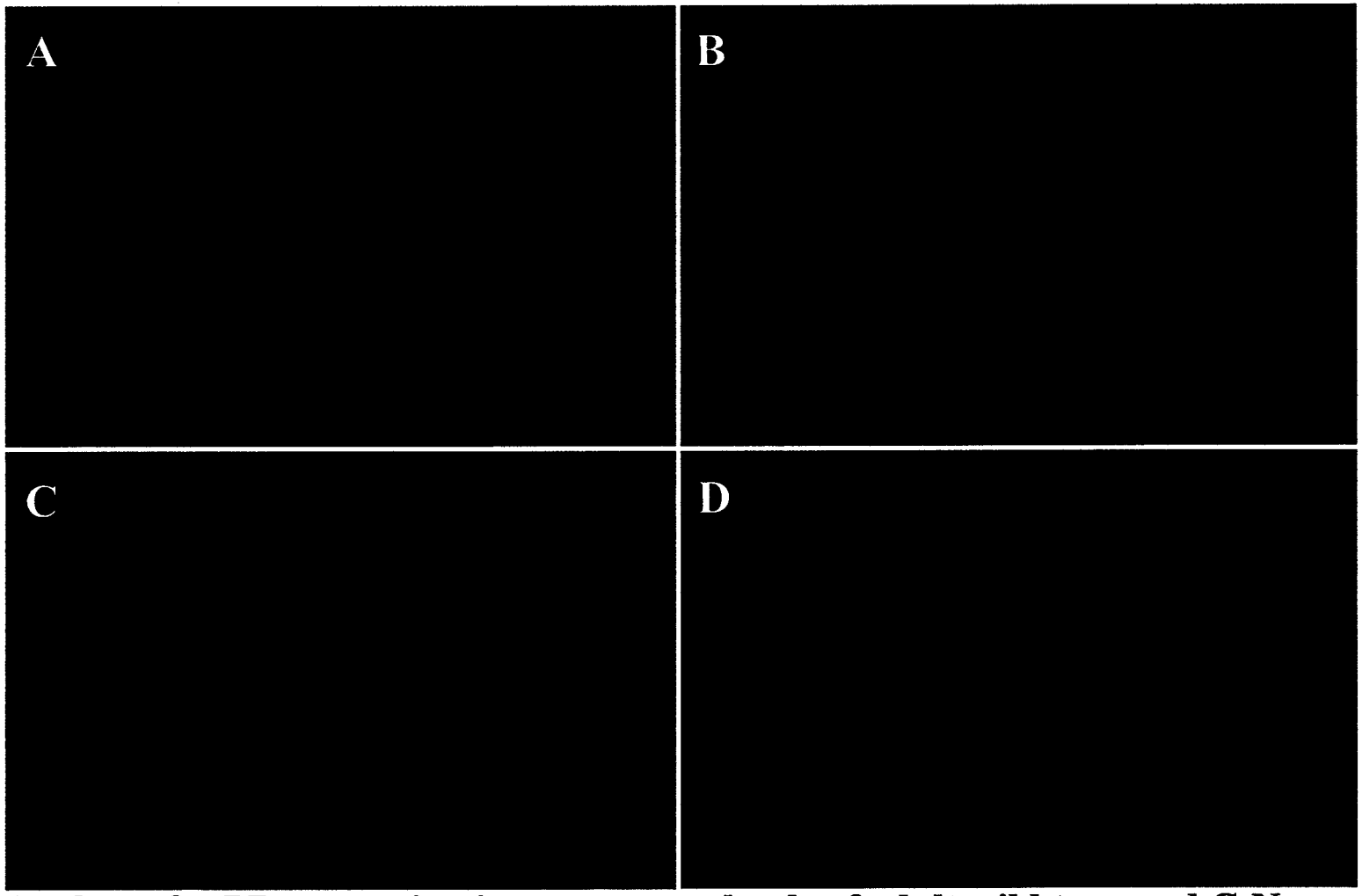

Figure 2. Analyses for PR expression in mammary glands of adult wild type and C-Neu transgenic mice. For detection of PR, an indirect immunofluorescence assay using an antibody prepared against mouse PR was used as described previously (Shyamala *et al.*, 1997). Panels on the left show DAPI (4', 6-Diamidino-2-Phenylindole) staining of nuclei which correspond to panels on the right which show FITC (Fluorescein Isothiocyanate) staining of PR in the same tissue sections. Panels A and B: adult wild type mouse. Panels C and D: adult C-Neu transgenic mouse.

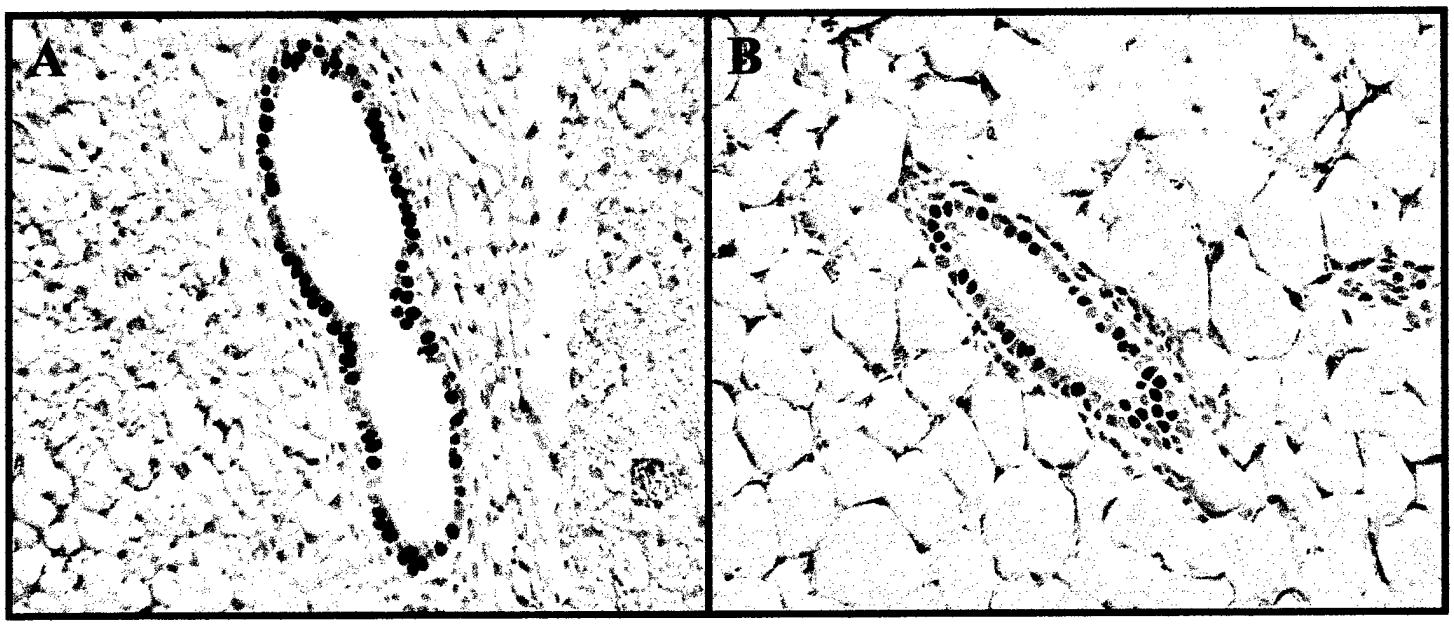

Figure 3. Analyses of PR expression in the mammary glands of adult wild type and C-Neu transgenic mice. For detection of PR, an immunoperoxidase assay using an antibody prepared against mouse PR was used. PR positive nuclei appear brown, and nuclei negative for the antigen appear purple-blue. Panel A: adult wild type mice. Panel B: adult C-Neu transgenic mice.

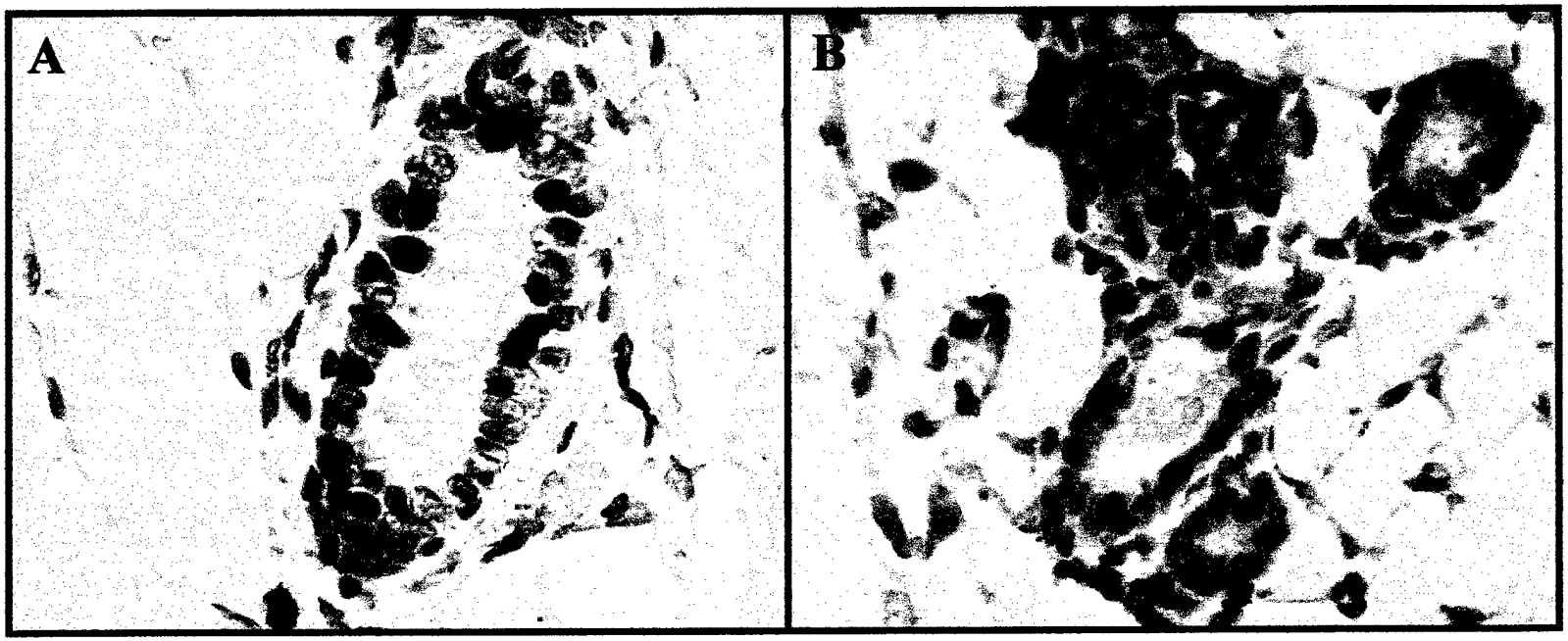

Figure 4. Analyses for PR expression in mammary glands of adult wild type and C-Neu transgenic mice. For detection of PR, an immunoperoxidase assay using a rabbit polyclonal antibody prepared against human PR (DAKO) was used. PR positive nuclei appear brown, and nuclei negative for the antigen appear purple-blue. Panel A: adult wild type mouse. Panel B: adult C-Neu transgenic mouse.

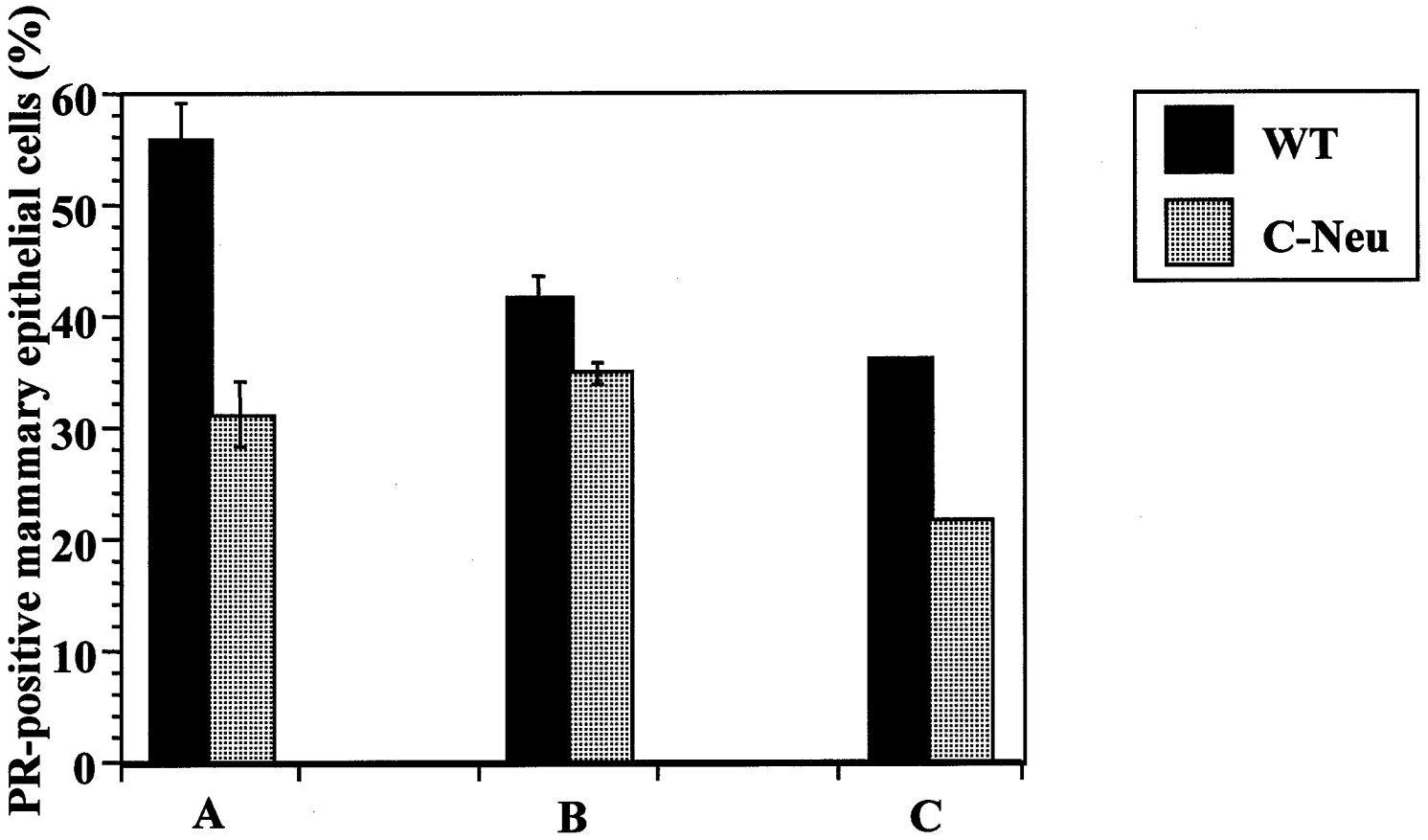

Figure 5. Quantitative analyses of PR expression in mammary glands of adult wild type and C-Neu transgenic mice. A: Immunofluorescence assay using anti-rabbit polyclonal antibody against mouse PR (Fig. 3). B: Immunoperoxidase assay using anti-rabbit polyclonal antibody against mouse PR(Fig. 4). C: Immunoperoxidase assay using anti-rabbit polyclonal antibody against human PR (DAKO) (Fig. 5).

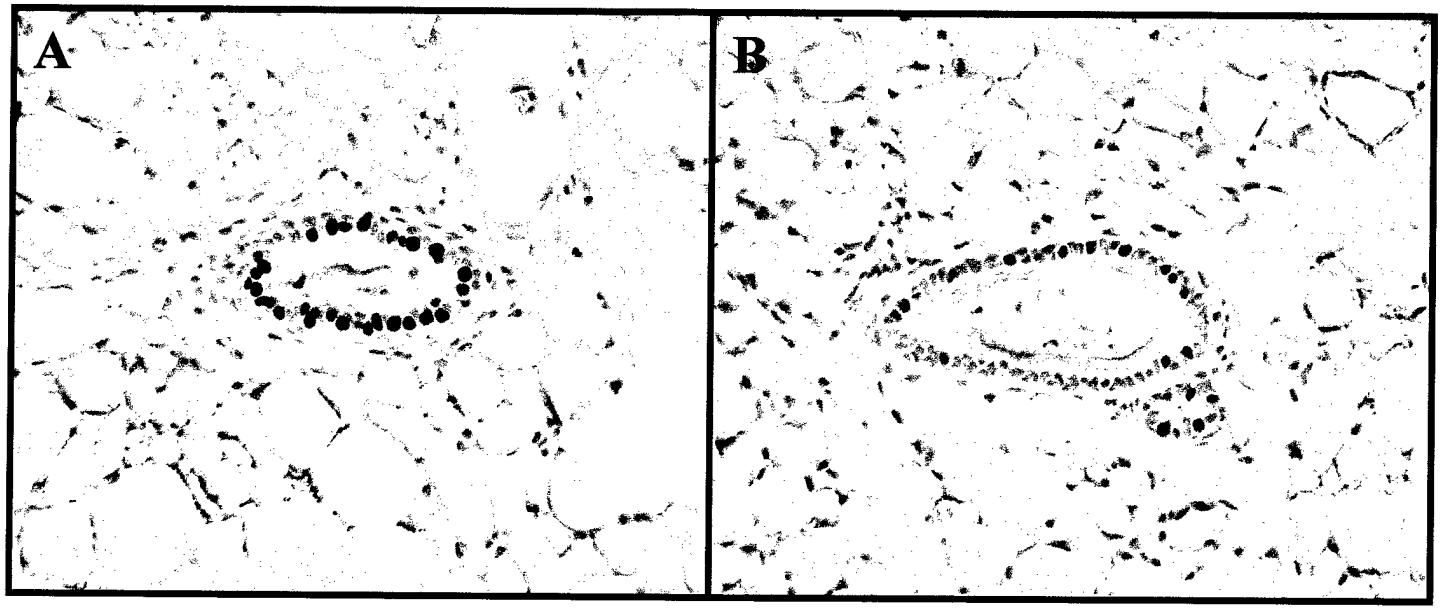

Figure 6. Analyses of PR expression in the mammary glands of pubertal wild type and C-Neu transgenic mice. Panel A: 6-week-old wild type mouse. Panels B: 6-week-old C-Neu transgenic mouse. Note that the intensity of immunostaining is reduced in the mammary epithelium of C-Neu transgenic mice. In mammary glands of C-Neu transgenic mice, the number of PR-positive cells is also reduced (26.3 $\pm$ 0.9%) as compared to those in wild type mice (34.5 $\pm$ 2.9%).